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Development of Cell Perfusion Device and ^{31}P NMR Spectrum Test[1]

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Abstract

To set up a device of cell perfusion, and use the method of cell perfusion -NMR to monitor the ^{31}P spectrum in cells. The result has shown the temperature and pressure and flow rate of the device were invariable relatively and could be controlled. The method of cell perfusion -NMR can monitor the ^{31}P spectrum online, and can Speculate changes of energy metabolism in cells under drug stimulation.

Keywords: Cell Perfusion device; ^{31}P NMR; Energy metabolism

1. Introduction

NMR spectrum can be used to observe the change of energy metabolism in cells and tissue harmlessly and continuously, and it is the main method to study energy metabolism the inside of cell [1, 2]. ^{31}P NMR has been used in all kinds of cell laboratory with its high sensitivity and simple NMR spectral line[3]. One difficulty of ^{31}P NMR to study cell metabolism is how to obtain a steady cell ^{31}P spectrum, because it needs a high density cell to obtain ^{31}P spectral line. Moreover, metabolism of the high density cell is quick, and cell metabolize normally can not be maintained for more than 3 hours, which requires the operation to be quick and accurate[4]. Huang Rong-qing and some other scientists once studied testing condition of ^{31}P NMR in the cell, such as the temperature and time etc, which influence the ^{31}P NMR spectral line [5]. The disadvantage is the tested must be done quickly and can not monitor online. If we will

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monitor the cell energy metabolism online for a long time, it needs to develop a cell perfusion instrument which can obtain high density, long time living cell. Moreover, this equipment must be used with NMR technology to test phosphorus' metabolism inside cell. Through monitor the signal change of ^{31}P spectrum online, we can Screen the effective ingredient of the medicine and Speculate changes of energy metabolism in Tumor cells.

2. Design and production of cell perfusion device

Keeping the cells with high density and living for a long time, the cells in the perfusion device must be germfree, maintained at 37℃, with a suitable pH and enough nutrient[6]. This perfusion device can keep the temperature in 37℃±0.2 with temperature controller and water cycle instrument. The pump provides power, and makes nutrient solution to flow continuously in the Perfusion system, to provide enough oxygen and nutrient. This instrument can observe pressure change of the experimental process with a indicate system, indicating the pressure change of the whole instrument through a U-pipe which contained mercury. This system can release the pressure when the instrument's pressure increased suddenly, making sure the whole instrument can run normally. Because the bubble in the pipe will break the balance of magnetic field, and affect experiment result, this instrument designed that the pump's front be in minus pressure, at the back be in plus pressure, which it can get ride of the bubble. The structure of cell perfusion device is shown in Figure1.

Making a cell perfusion device according to Figure1, stick the NMR pipe which contained with cells in NMR spectrometer and taking online test. The performance showing that the temperature and pressure and flow rate of the device were invariable relatively and could be controlled [7].

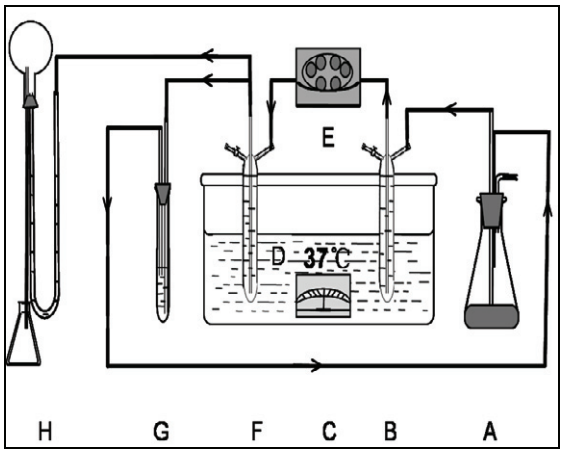

Fig. 1 Cell perfusion device structure

A. culture bottle; B. tube 1; C. Temperature control instrument D. The thermostatic water tank; E. Peristaltic pump; F. tube 2; G. Cell-NMR tube ; H. U tube

3. Cell persusion -nmr experiment

3.1 Apparatus and reagents

NMR, ECX-500,500MHz, JEOL, Japan; Cell Perfusion device. Own development; Agarose, The United States of America FMC company; MEM Culture medium, The United States of America hyclone

company; MCF-7 breast cancer cells, cell library of Shanghai.

3.2 Experimental methods and conditions

The MCF-7 cells ($0.8\sim1\times10^8$cells) were collected by trypsin into a centrifuge tube with 1500 r/min giving 0.4ml Cell suspension. The Cell suspension was maintained in a 37℃ water bath and mixed with 0.4 ml low temperature-gelling agarose which dissolved in PBS (1.8%). The agarose-cell mixture was extruded with stable pressure into a 5mm NMR tube containing MEM Culture media. The NMR tube containing the agarose-encased cells were then placed inside the NMR magnetic and perfused at the rate of 0.4 ml/min. The agarose-encased cells were tested by ^{31}P NMR after perfusion one day. The NMR conditions: at = 0.25s, sw = 5000, pw = 8.9us, nt = 1200, d t = 1.75s, NMR tube does not rotate.

Experimental results have shown that the ^{31}P NMR spectra of cells can not be measured in at low dense cells (1 x 10^7 cells) and the long time of process. It is difficult that the ^{31}P NMR spectra of high dense cells (1 x 10^8 cells) be measured under the condition of not using perfusion culture. Because adherent cells of high density usually takes more than 2 hours on processing and testing of cell, and the cells will occur the metabolic change in the process of the processing and testing. However, using the same amount of high density cells which continue perfusion culture, usually 15-25 hours, can measure ^{31}P NMR spectroscopy in cell(Figure 2). From spectrum 2 know that the peaks are identified as phosphomonoesters (PME, 5.80 ppm); inorganic phosphate (Pi, 4.70 ppm); phosphocreatine (PCr, 0.0 ppm); γ-ATP phosphate (-2.48ppm); α-ATP phosphate (-7.57 ppm), and β-ATP phosphate (-16.73ppm). The experiment results in accord with ^{31}P NMR spectrum in the literature [2, 8].

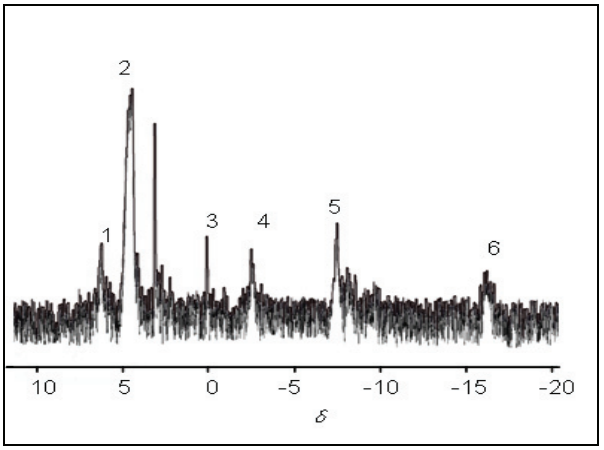

Fig. 2 ^{31}P NMR spectrum of high dense cells which continue perfusion

1. phosphomonoesters（PME）; 2. inorganic phosphate（Pi）; 3. phosphocreatine（PCr）; 4. γ-ATP; 5. α--ATP; 6. β-ATP

Cell perfusion - NMR method show that the stability ^{31}P-NMR spectrum must be high concentration cell and perfusion culture. At the same time, cell processing to quickly and control the temperature at about 10 ℃ to reduce the cell metabolism, which more advantage to ^{31}P testing.

3.3 NMR on-line monitoring cell under drug perfusion

By above the experimental method and conditions, ^{31}P spectrum of living cells(1 x 10^8 cells) were

tested after perfusion culture one day. The next ,used containing 5 x 10^{-4} mol / L cantharidin medium continued perfusion culture, and monitored ^{31}P NMR spectrum of MCF-7 cells on-line under drug perfusion at 2, 4, 6 hours. As shown in figure 3.

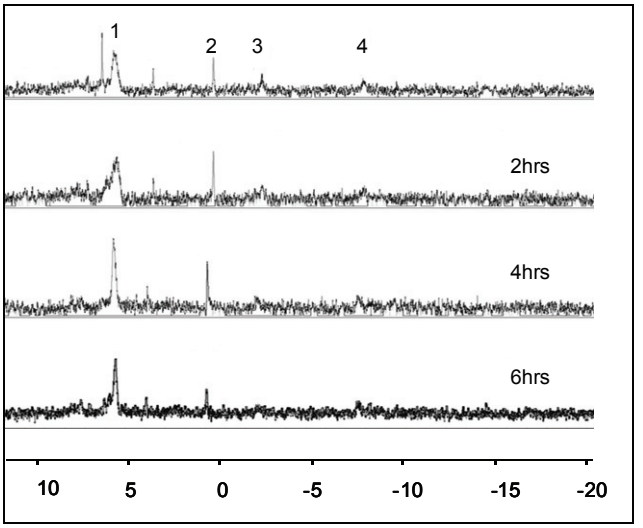

Fig. 3 ^{31}P NMR spectrum under drug stimulation

1. inorganic phosphate（Pi）; 2. phosphocreatine（Pcr）; 3.γ-ATP; 4.α-ATP;

From the Fig. 3 shows, after 2 hours, intracellular(Pi, 4.70 ppm,) and extracellular(Pi, 5.58 ppm,) inorganic phosphorus change to a width of inorganic phosphorous peak gradually, and γ- ATP and α- ATP peak does not change obviously. It suggests that drugs have effect in cells. After 4 hours, intracellular and extracellular inorganic phosphate peaks have combined for an inorganic phosphate peaks, and γ - ATP and α- ATP peak has weakened. After 6 hours, γ - ATP and α- ATP peak more weakly, and inorganic phosphorous peak narrows, which indicate that metabolism change of the breast cancer cell speeds up. Cells tend to death with the monitoring time growth, ATP will disappear gradually and inorganic phosphorus peak exists only. Drug perfusion experiment has shown that the method of cell perfusion NMR can monitor growth status and energy metabolism changes online at different time of the cell.

4. Result and discussion

The cell perfusion instrument can obtain high concentration, long time living cells, and control the pressure and flux well. By using circle perfusion cultivating, this instrument overcome a defect that Culture medium was not fully used. The cell Perfusion-NMR can not only obtain steady cell ^{31}P NMR spectrum, it can monitor cell's growth status and energy metabolism changes online under drug stimulation. Cell Perfusion-NMR can provide a way to Screen the effective ingredient of the medicine. In this experiment, the NMR instrument only use 5mm NMR test tube, the number of cells fixed in this tube is limited, which influence the signal collection of cell ^{31}P. It made part of cell ^{31}P NMR spectral line's tip be weak or disappear. If we use 10mm NMR test tube, we can well observe cell's growth and metabolism. Because instrument is limited, related research is less in our country. In foreign country, they had used ^{31}P NMR inside human body to test Anti-tumor medicine with suitable dosage and time [9]. Cell

Perfusion-NMR is harmless to biologic sample. It can also observe information of the cell PME, Pi, ATP and other compound contained phosphorus. It has a very good foreground, it deserves us to study further.

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